SYNOPSIS

(1) TITLE OF THE SYNOPSIS

"QUANTUM DOT BIOCONJUGATES: SYNTHESIS CHARACTERIZATION AND BIOMOLECULAR INTERACTION OF MEDICINALY IMPORTANT BIOMOLECULE"

(2) INTRODUCTION

One of the fastest moving and most exciting interfaces of nanotechnology is the use of quantum dots (QDs) in biology. The unique optical properties of QDs make them appealing as in vivo and in vitro fluorophores in a variety of biological investigations, in which traditional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of multiple signals. The ability to make QDs water soluble and target them to specific biomolecules has led to promising applications in cellular labelling, deep-tissue imaging, assay labelling and as efficient fluorescence resonance energy transfer donors. Despite recent progress, much work still needs to be done to achieve reproducible and robust surface functionalization and develop flexible bioconjugation techniques.

Recently, quantum dots [CdS, CdSe, ZnS, CdTe, etc.] have attracted tremendous interest as luminescent probes in biological and medical researches due to their unique optical and chemical properties.[1] Compared with traditional dyes and fluorescent proteins used as imaging probes, QDs have several advantages, such as tunable emission from visible to infrared wavelengths, broader excitation spectra, high quantum yield of fluorescence, strong brightness, photostability, and high resistance to photobleaching.[2-3] However, the potential applications of QDs in biology and medicine have been limited due to their cytotoxic effects.[4] QDs contain

toxic components such as cadmium (from cadmium chalcogenide-based QDs) or lead (from lead chalcogenide-based QDs). Cd^{2+} and Pb^{2+} can be released from QDs, which would kill the cells.[5] Therefore, to enhance stability; the surface modification of QDs is required. For example, biomedical applications require high-quality water soluble and nontoxic QDs. So far, numerous surface modifications of QDs have been explored, including the attachment of mercaptoacetic acid[6], mercaptopropionic acid[7], mercaptobenzoic acid[8], and biocompatible and chemically functionalizable inorganic shells, such as silica or zinc sulfide[9]. All of these coatings can ensure the water solubility of QDs, but they are unable to enhance biocompatibility. Therefore, further coating with suitable water-soluble organic ligand/biomolecules is necessary to enhance the biocompatibility of QDs. To this end, QDs have been covalently linked with biorecognition molecules such as biotin[10], peptides [11], bovine serum albumin[12], transferrin[13], antibodies[14], and DNA.[15]

(3) REVIEW OF THE EARLIER WORK

Synthesis, Characterization, and quantum confinement has been largely studied by Haram and coworkers.[16-17] Recently, Pal *et al.*[18] investigated covalently conjugation of CdS Nanocrystal with α-Chymotrypsin. One-pot synthesized neoglycoconjugates with a reactive thiol group is reported for functionalization with carbohydrates for solubilization and stabilization of CdSe–ZnS quantum dots in aqueous solution. Three different sizes of quantum dots (QDs) with lactose, melibiose, and maltotriose on their surface have been utilized, for the first time, for lectin detection through agglutination assay[19]. Shastri and coworkers[20] reported a quantum dot (QD)-tailored western blot analysis for a sensitive, rapid and flexible detection of the nuclear and cytoplasmic proteins. Quite recently, a new class of zinc oxide quantum dots (ZnO QDs) was investigated as nanoprobes for targeting cancer cells in vitro.[21] ZnO nanoparticles were synthesized using conventional sol–gel method and encapsulated using trimethoxy aminopropyl silane. Transferrin, the ligand targeting the cancer cells, was conjugated to the ZnO QDs. In vitro imaging studies using MDA-MB-231 showed the biocompatible ZnO nanoprobe selectively

binding to the cell surface receptor and internalizing through receptor-mediated endocytosis. Time-lapsed photobleaching studies indicate the ZnO QDs to be resistant to photobleaching, making them suitable for long term imaging purpose.

A simple, rapid, sensitive and specific detection method for silver ion was proposed by Wang *et al*.[22] the fluorescence intensity of CdS QDs is linearly proportional to silver ion concentration. It was found that the concentaration of silver ion from 2.0×10^{-8} to 1.0×10^{-6} mol/L with a detection limit of 5.0×10^{-9} mol/L is directly proportional to the fluorescence intensity of CdS QDs. Colloidal Synthesis of CdS/HgS/CdS quantum dot quantum well nanocrystals and CdS/HgS/CdS/HgS/CdS double quantum well nanocrystals stabilized with polyphosphates have been investigated by photoelectron spectroscopy with tuneable synchrotron radiation.[23] An ambient synthesis of CdS QDs with broadly tunable particle size by sulfur source of H_2S and a new capping agent R-2-thiotetrahydrothiazole-4-carboxylic acid in situ produced from the reaction of L-cysteine amino acid with carbon disulfide in water.[24] Surface passivation and stabilisation of CdS@MPA cores by a broader band gap material, ZnS, led to improved luminescence intensity. CdS@MPA and CdS/ZnS@MPA QDs show high photochemical stability and hold a good potential to be applied in optoelectronic devices and biological applications[25]. Production of core shell CdTe/CdS quantum dots with three different stabilizer TGA troponin and glutathione has been done by Liu *et al*.[26] Fluorescent semiconductor quantum dots as selective ion probes in aqueous samples was done by Rosenzweig *et al*.[27]

Djikanović *et al.* [28] demonstrated that CdSe QDs bind typically to cellulose and lignin in the cell wall of Picea omorika branch. Respectively,binding to lignin and cellulose are achieved by interaction with the chains of C=C and C-C alternating bonds and interaction with the OH groups. Application of QDs' as pH probe has been studied by. Gao *et al*.[29]

Hasegawa *et al.* [30] demonstrated that $CHPNH_2$-QD complexes which were uniformly internalized into the cells without being aggregated. Therefore, $CHPNH_2$ nanogel has

high potential for use in long-term live cell imaging. The interaction of QDs with cells was fruitfully controlled by the amino group content of the $CHPNH_2$ nanogel.

Howarth *et al.*[31] demonstrated a technique to track endogenous cell-surface proteins without cross-linking by purifying monovalent antibody-QD conjugates.

Yu *et al.* [32] reported the use of GSH-TGA-QDs-ND-1probes to label colorectal cancer cells CCL187. They fabricate QDs, which were conjugated with monoclonal antibody ND-1 for specific reaction with antigen LEA.

(4) OBJECTIVES OF THE STUDY

The fundamental objectives of the present investigations are as follows:

(i) Synthesis and characterization of CdS, CdSe, CdTe, ZnSe, ZnTe quantum dot nanoparticles.

(ii) To study the interaction of thiol drugs and thiol functional biological molecule onto quantum dot surface.

(iii) To investigate the immobilization of insulin on PEG functionalized quantum dot surface.

(iv) To study covalently coupling folic acid (FA) onto the water-dispersible PEG-coated quantum dots (PEG-QDs) to produce FA-coupled PEG-QDs (FA-PEG-QDs).

(v) Investigation of surface immobilization of antibiotics and their biological activity.

(5) NOTEWORTHY CONTRIBUTIONS

Quantum dots are the vital field of research these days due to its wide range of application in the field of biology, Physics, Chemistry and all other fields of science. In this context we have studied the facile route of synthesis and charachteristion of thiol capped CdS quantum dot nanoparticles. The optical properties, size, shape and crystallinity of of thus synthesized CdS quantum dots have been investigated. Figure 1-2 shows HRTEM image of cysteine (Cys) and thioglycolic acid (TGA) capped CdS

quantum dots the selected area electron diffraction patterns of both thequantum dots are shown in figure 3&4. These prelimnary studies encourage furthering exploring the present proposal.

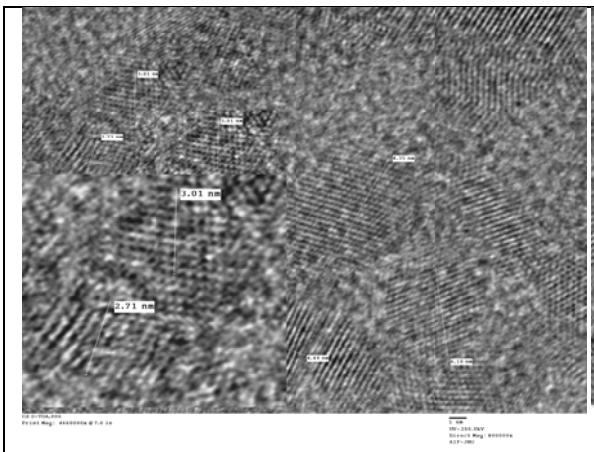


Fig.1: HRTEM image of Cys-capped CdS quantum dots

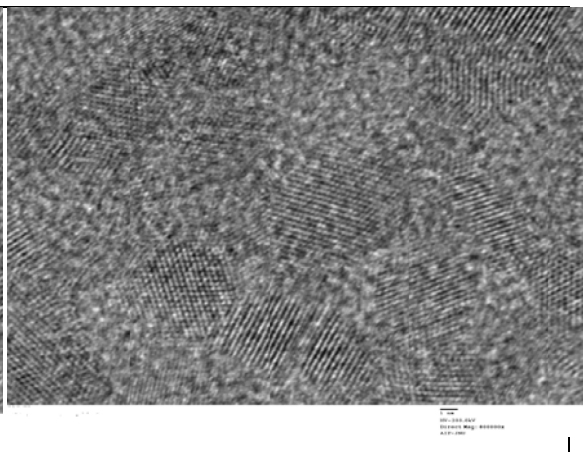


Fig.2: HRTEM image of TGA-capped CdS quantum dots

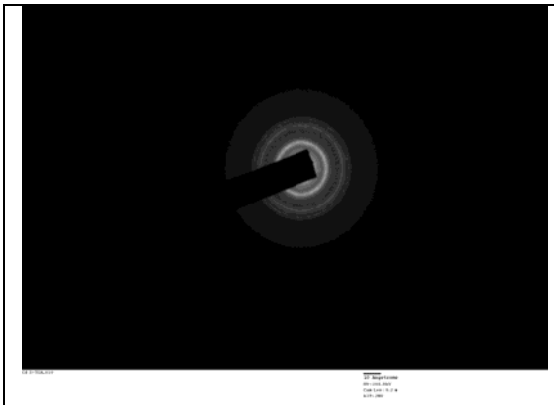


Fig.3: SAED pattern of Cys-capped CdS quantum dots

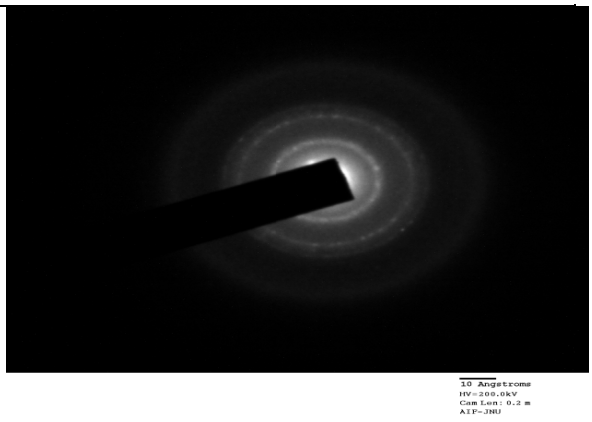


Fig.4: SAED pattern of TGA-capped CdS quantum dots

(6) PROPOSED METHODOLOGY

Methodology of the entire work has been distributed in to following sections which are as follows.

I. Synthesis and Characterization of CdS, CdSe, and CdTe Quantum dot nanoparticles.

Synthesis of mercaptoacetic acid-coated CdS quantum

The carboxyl-stabilized CdS quantum dot will be synthesized by arrested precipitation at room temperature in aqueous solution using mercaptoacetic acid as the colloidal stabilizer. A stirred solution of $CdCl_2$ (5 mM) in distilled water will be added thiol capping agents (**Chart-1**) maintaining pH 7 with 1 N NaOH. The mixture deaerated by N_2 bubbling for about 30 min, will be added freshly prepared 5 mM Na_2S with rapid stirring. The solution turned yellow shortly after the sulfide addition due to the formation of CdS quantum dot. CdS QD will be separated from reaction by-products (sodium salt) via precipitation by the addition of acetone (4 ml of acetone per milliliter of nanocrystal solution). Then the precipitate will be isolated by centrifugation and dried in a freeze dryer. The prepared powder CdS QD will be finally redispersed in water to obtain a clear colloidal solution with excellent stability.

Synthesis of CdSe and CdTe quantum dot.

Two synthetic methods will be adopted:

Method-1

The CdSe quantum dot will be synthesized literature method using following protocols:

1. Prepare a 1M Stock solution of TOP (trioctylphosphine): selenide/telluride (TOP: Se/Te) by dissolving Se/Te into 100 ml of TOP.

2. Add 170-250 μl of $CdMe_3$ and 3.5-4 ml of 1M TOP: Se to about 15 ml of TOP.
3. Mix under inert atmosphere in a glove box.
4. Load into syringe equipped with a large-gage needle for injection. Store in the glove box until **step 9.**
5. Load 20-30 g of TOPO (trioctylphosphine oxide) into 100 ml three nacked flask.
6. Heat TOPO to 150-160 ^{o}C for 2 hours under vaccum while stirring.
7. Backfill with inert gas (typically argan or nitrogen).

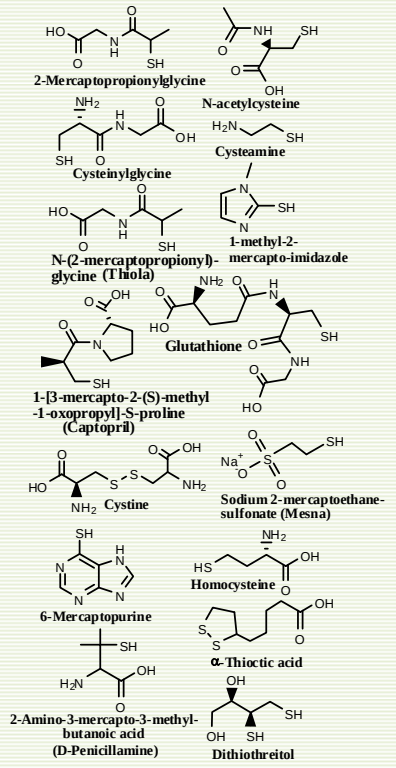


CHART-1

8. Raise the temperature to 300-350 ^{o}C in preparation for precursor injection.

9. Remove the flask from heating source. Retrive the syringe from inert chamber (glove box) and quickly inject the syringe content into the 100 ml flask.

10. Keep temperature below 200 ^{o}C for few minutes (to avoid growth) and take an absorption spectrum. The spectrum should show resolved feature with the peak of the frist transition (band edge absorption) usually located approx 490 nm.

11. Raise the temperature to 280-300 ^{o}C, these higher temperature allow growth and anneling of the quantum dots.

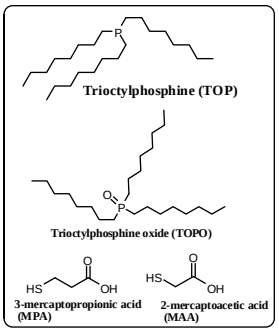
12. During growth, periodically remove samples and take their absorption spectra. Monitor the position of the first absorption peak and its relative width; this is usually indicative of a sample's size distribution.

13. Once the location of the first absorption peak reaches a wavelength indicative of a desired size, drop the temperature to below 100 ^{o}C to arrest crystal growth.
14. Store the growth solution in a mixture of butanol and hexane (or toluene).

15. The water-soluble quantum dot will be prepared by ligand exchange between TOPO and thiol ligands

Method-2

CdSe/CdTe QD will be synthesized in aqueous phase by two steps according to the reported method with minor modification.[33] Briefly, selenium/tellurium powder and 0.1 g $NaBH_4$ will be added to 10 ml ultra pure water under nitrogen atmosphere to from blak mixture, which will then stirred for 8-9 hours in an ice bath. After the black color disappeared and white $Na_2B_4O_7$ is produced, the supernatant containing NaHSe/NaHTe will be separated from the mixture and used as precursor for the preparation of CdSe/CdTe QDs. The $CdCl_2.2.5\ H_2O$ and thiol capping agents (**Chart-1**) will be dissolve in water with adjusting pH 9.0 using 1.0 M NaOH. To the deoxygenated mixture by bubbling nitrogen, freshly prepared NaHTe solution will be added (final molar ration: Cd^{2+}/Se^{2-} or Te^{2-}/thiol ligand = 1:0.5:2.5). After stirring for 20 min at room temperature, the mixture will be refluxed to produce desired size by controlling refluxing time.

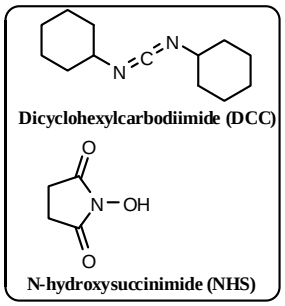


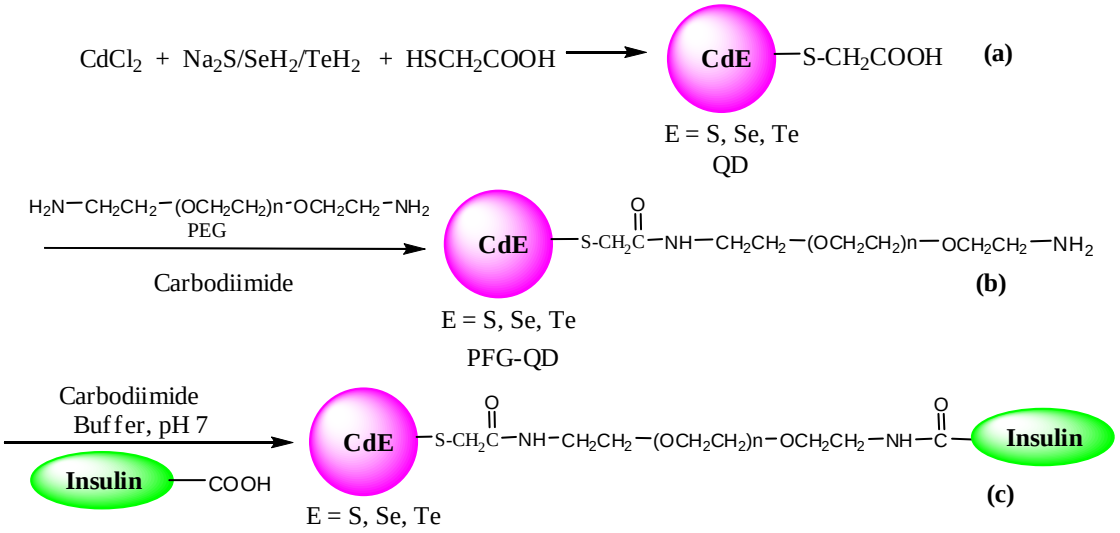
Characterization of Quantum dots:

All the synthesized quantum dots will be characterized by UV-visible, fluorescence emission, TEM, and XRD analysis.

II. Immobilization of insulin on PEG functionalized quantum dot surface.

Immobilization of insulin on QD will be performed in two steps. First, quantum dot (QD) will be reacted with amino-terminated polyethylene glycol (PEG) to introduce amine groups on their surface. For this, QD will be redispersed in aqueous solution containing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and stirred for 4 h to activate the carboxylic acid groups on the surface. Then, an excess amount of amine-terminated PEG will be added to the solution, which will be stirred for 24 h to obtain PEG-grafted QDs (**Scheme 1b**). An excessive amount of PEG will be used to suppress the cross linking reaction on the surface and keep free amine groups at one end of the PEG chain after the reaction. Prepared PEG-grafted QD is isolated via repeated centrifugation and finally dried in a freeze dryer. In the second step, insulin will be immobilized on the surface of PEG-grafted QD as follows: insulin will be dissolved in phosphate buffer solution (2 mg/ml, pH 7.4) followed by the addition of a small amount of 0.1 N HCl. Then, 2% w/v water-soluble EDC and NHS will be added to the solution, which will be incubated at 4°C for 5h to activate the carboxylic acid groups of the chain. Then, PEG-grafted QD (5 mg/ml) were suspended in phosphate buffer solution (pH 7.4) with vortexing. This PEG-grafted QD suspension will be mixed with the insulin aqueous solution and stirred gently overnight at room temperature to obtain insulin-immobilized PEG-grafted QD as shown in **Scheme 1**. Insulin immobilized QD will be isolated by repeated centrifugation and stored in phosphate-buffered saline (PBS) at pH 7. All conjugation reactions, unless otherwise noted, will be carried out in the dark under a N_2 ambient environment.





Scheme-1

III. Covalently coupling folic acid (FA) onto the water-dispersible PEG-coated quantum dots (PEG-QDs).

Synthesis of CdSe/ZnS QDs and water-dispersible FA-MAA-QDs.

The monodisperse hydrophobic CdSe/ZnS QDs with a diameter of about 5 nm will be synthesized as described[34-35], using trioctylphosphine oxide as the solvent in the synthesis. The water-dispersible FA-MAA- QDs will be prepared via ligand exchange. Briefly, a mixture of MAA and dried CdSe/ZnS QDs will be first stirred vigorously for1h to prepare MAA-capped QDs via ligand exchange. Then the MAA-capped QDs will be activated with DCC and NHS for 30 min. Finally, the FA will be added and stirred overnight to produce FA-MAA-QDs.

Preparation of PEG-QDs

The water-dispersible PEG-QDs will be prepared based on the method of Dubertret *et al.*[36] with some modifications. We will use a molar ratio of 30% mPEG-1000 and 70% aminoPEG-2000 phospholipids. We will dissolve the PEG and QDs at a 300:1mol/L ratio in 10mL chloroform. The chloroform will be evaporated over a 30-min period using circumrotate evaporation instrument, and the residue will be

dried by heating to 80 °C for 10 min. The dry film will be resuspended by adding hot water (about 80 °C), followed by filtering with a 0.1-μm syringe filter to remove the large, hollow PEG micelles. The emulsion will be centrifuged at 500 rpm for 2 h with a Beckman ultracentrifuge and resuspended the pellet in 1mL ultrapure water. This generated clear and stable PEG-QDs.

Preparation of FA-PEG-QDs

Briefly, FA will be activated with DCC and NHS at a 1:2:2 mol/L ratio in DMSO for 12 h and filtered with a syringe filter (pore size 0.2 μm). The PEG-QDs will be added to the solution at a molar ratio of 10:1 (FA: PEG-QDs). After reacting for 4h at room temperature in the dark, the reactants will be dialyzed against a 0.05 mol/L sodium bicarbonate buffer (SpectraPor6; molecular weight cutoff 1000). It is important to note that the addition of DCC and NHS to folate forms a highly reactive intermediate (FA-NHS), which can subsequently react with the free amino group present in PEG-QDs to form the resulting FA-PEG-QDs, as shown in Fig. 1.

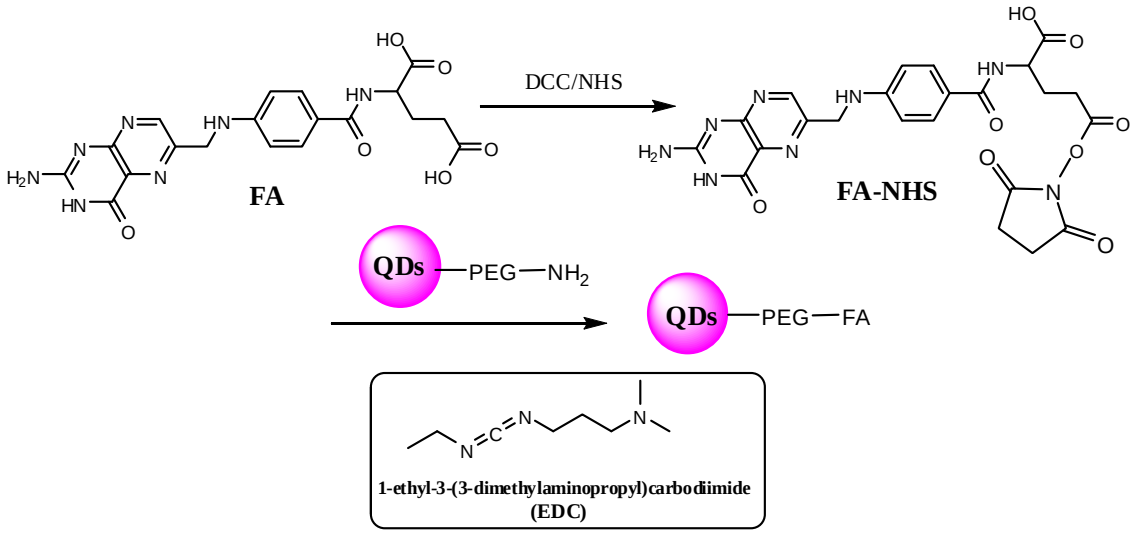
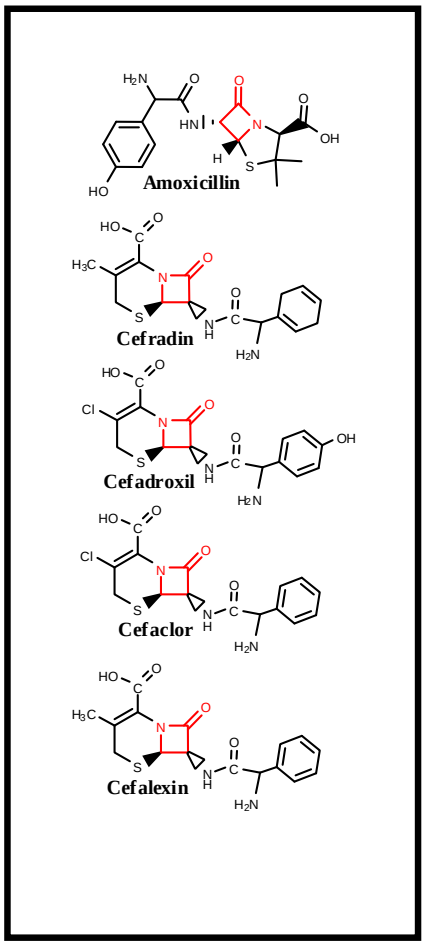


Fig.1.Schematic route for preparation of FA-PEG-QDs.

IV. Investigation of surface immobilization of antibiotics and their biological activity.

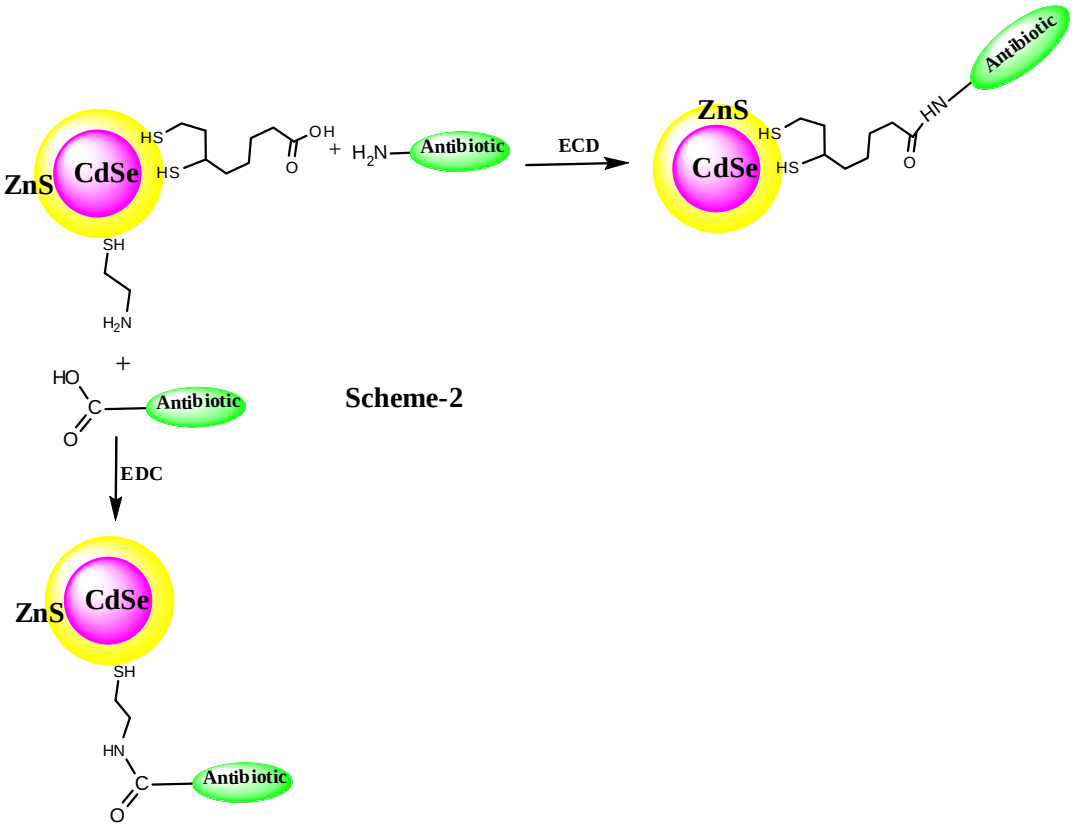
β-Lactam antibiotics are (beta-lactam antibiotics) a broad class of antibiotic agents, consisting of all antibiotic agents that contain a β-lactam nucleus in their molecular structure. This includes penicillin derivatives, cephalosporins, monobactams, and carbapenems.[37] Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Up until 2003, when measured by sales, more than half of all commercially available antibiotics in use were β–lactam compounds.[38]



Quantum dots rendered water soluble for biological applications are usually passivated by several inorganic and/or organic layers in order to in order to increase fluorescence yield. However, these coatings greatly increase the size of the particle, making uptake by microorganism impossible. The possible mechanism through which nanocrystals could pass through bacterial cell walls are nonspecific diffusion, nonspecific membrane damage, and specific uptake. It is find that adenine- and AMP- (adenosine monophosphate) conjugated QD are able to label bacteria only if the particles are < 5 nm in diameter.[39] Therefore, it is proposed to investigate the antimicrobial activity some antibiotic-conjugated quantum Dots.

Different size bare CdSe and CdSe/Zns core shell quantum dots will be synthesized as described previously. Size distribution will be determined from fluorescence emission spectra and high-resolution transmission electron microscopy (HRTEM).

Bare CdSe QDs will be synthesized, characterized, solublized with mercaptoacetic acid, and conjugated to primary amine-containing antibiotics using the activator 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. In order to conjugate the QDs through the carboxylic group of antibiotics, the amine functionalized QD will be synthesized using 2-aminoethanethiol. The pH of all solution will be adjusted to neutral. Core shell QDs will be produced after synthesis of CdSe QDs by capping with ZnS.[40] Thus synthesized core shell QDs will be solubilized/functionalized by dihydrolipoic acid (DHLA) and will be conjugated with antibiotics using EDC coupling agent. The complete procedure is summarized in **scheme-2**.



Scheme-2

V. The antibacterial activity measurements.

The antibacterial activity of the QD-conjugate antibiotic compounds will screened against some selected strains of human pathogenic bacteria, viz., ***Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Citrobactor sruendi,***

Bacillus megaterium, Proteus vulgaris, Micrococcus luteus and Pseudomonas aeruginosa by applying the disc diffusion assay. The free (non-conjugated) antibiotics will be used as standard. Typically, sterilized melted nutrient agar medium (45°C) will be poured at the rate of 15 ml into each Petridis (90 mm). After solidification of the medium, the agar media will be inoculated with 0.5 ml of liquid culture for 24 h. Prepared discs of samples will be placed gently on solidified agar plates, freshly seeded with the test organisms with sterile forceps. A control disc will also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent respectively. The disc bearing plates will be incubated within 30 minutes at 37°C for 24 hours. Inhibitory activity will be measured (in mm) as the diameter of the observed inhibition zones.

(7) EXPECTED OUTCOME OF THE PROPOSED WORK

The utility of QDs in biological system is requiring controlled size and their biocompatibility. In principle, the binding of biomolecule to the quantum dot surface and their fate into the biological cells are the key factors. A systematic investigation on the thiol capping, most of which are drugs will bring out usefulness of quantum dot nanomaterials on the probing biological process. For example, the concentration and the distribution of the captropril, an anti-hypertensive drug in the blood and the organs can be evaluated with their QD-captopril conjugate fluorescence.
It is of prime important to known the ultimate fate of insulin in the human organism. Obviously, insulin is constantly being eliminated; otherwise cumulative effects manifested by periods of severe hypoglycemia would be observed even in normal subjects. As is well known, however, the blood sugar level is maintained remarkably constant in normal persons. At least two different methods of disposing of insulin appear possible: one by excretion in the urine or possibly feces (via bile) and the other by destruction in the body. The first route that of excretion, would not appear to be of major importance. The elimination of protein molecules as large as that of insulin (molecular weight 35,000) seldom takes place to any significant extent in the normal kidney and probably not in the bile or feces. In vitro fluorescence probing of the QD-insulin conjugate will provide new insight to understand the fate of insulin in vivo.

The photophysical properties of Folate-conjugated quantum Dot will results on the investigation of either FRET or single oxygen generation process. The fluorescence quenching or enhancement of fluorescence intensity of QD upon the conjugation of Folic acid will revealed the application of the system is biology.
The cyto-toxicity of the QDs are long debated. It will be interesting to find results on the antibacterial screening of commercially available antibiotics conjugated with QDs. Several questions will be answered after findings such as:

- Are quantum dot toxic to bacteria, if so, is this related to the presence of heavy metals?
- Must quantum dot be degraded in order for the toxicity to be seen?
- Does the size of the quantum dots matter? If so, is that related to the redox properties of the particles?
- What are bacterial mechanisms for sequestering or eliminating quantum dots?

(8) RESEARCH PUBLICATION

An Ambient Route for Synthesis of Thiol-Capped CdS Quantum Dot

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Communicated to Chemical Physics Letters,

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